

Review of xanthan gum production from unmodified starches by *Xanthomonas campestris* sp.

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Abstract

Many attempts were reported to optimise variables in xanthan gum fermentations, i.e. the nutrient composition and feeding technique, temperature, pH, agitation, and adding antifoam. All shows some improvement in the area studied. Other substrates were also tested, such as hydrolysed rice, barley and corn flour, acid whey and sugarcane molasses, etc., but glucose is still the best in-term of the product yield, supply, and the product quality. Sufficient studies of the unstructured kinetics and the structured kinetics models were described in batch processes but the continuous kinetic model is insufficiently cared. Looking at the conventional xanthan production, mixing is the main problem occurred in batch fermentation as the produced broth during the production stage is very viscous, therefore mixing requires considerable balance between cell disruptions and oxygen transfer. Giving the support, e.g. cotton wool and fabric, for microorganisms to adsorb may ensure the nature physical separation between microorganisms and the liquid phase containing nutrients and products. However, the specific xanthan productivity was reported low due to relatively low cell viability. The problem of the limited oxygen transfer suggests that a new bioreactor design is required. The design strategy could be by freely moving of the liquid media and air passing through the porous fibrous matrix, therefore should ensure a good contact between cells that adsorb onto the fibrous matrix support and nutrients. This strategy should improve oxygen transfer, and may increase the reaction rate and reduce the fast growing of mutation. Using ultrafiltration was reported save up to 80% of the energy is required for recovering of xanthan gum.

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1. Introduction

In response to environmental concerns, some industries which have previously used non-degrading polymers in their products and raw materials have looked closely at the possibilities of using materials that are “greener” and environmentally friendly. Polymers can be manufactured from petrochemicals, plants, or animals based raw chemicals. Petrochemical based polymers are the less preferable choice compared to biopolymers collected or manufactured from plant or animal sources especially for use in consumer products. Despite the increasing collection and extraction costs, and volatile market prices of plant and algal gums (biopolymers) it is suggested that the industrially produced biopolymers (e.g. modified starches, celluloses, and microbial polysaccharides) could be a suitable alternatives.

Xanthan gum has discovered in the late 1950s by US Scientists and is the first biopolymer produced industrially. The natural source of the polysaccharide came from a cabbage plant bacterium, known as *Xanthomonas campestris*. It was not until 1969 that the FDA issued the final approval for the use of xanthan gum in food products. The demand for xanthan gum produced by *Xanthomonas campestris* sp. has increased steadily every year and is estimated to grow continuously at an annual rate of 5–10%. Commercial production of xanthan gum uses glucose as the substrate, and generally batch production instead of continuous production due to the batch process having been proven to work successfully. However, increasing market price and demand suggests that glucose may no longer economic for the raw material, while using batch processes may also limit the capacity. It is therefore the purpose of this review to investigate alternatives to economically produce xanthan gum.

2. Application of xanthan gum

Xanthan gum is widely used in a broad range of industries, such as in foods, toiletries, oil recovery, cosmetics, as

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Nomenclature

C_N	nitrogen concentration (g/l)
C_{O_2}	oxygen concentration
$C_{O_2}^*$	saturated oxygen concentration
C_P	product concentration (g/l)
C_S	substrate concentration (g/l)
C_{S_m}	maximum substrate above which growth is completely inhibited (g/l)
C_X	biomass concentration (g/l)
C_{X_m}	maximum concentration of biomass (g/l)
C_ξ	other special adverse components or inhibitors concentrations, e.g. C_{CO_2} , C_{Poison} (chemical poison concentration) (g/l)
f	function particular to the system used
k_{La}	oxygen mass transfer coefficient (s^{-1})
k_P	maximum specific production rate ($l\ g\ S^{-1}\ g\ X^{-1}\ h^{-1}$)
k_X	maximum specific growth rate ($l\ g\ N^{-1}\ g\ X^{-1}\ h^{-1}$)
K_S	saturation or Monod constant (g/l)
m_{O_2}	dissolved oxygen consumption coefficient ($mol\ O_2\ g\ X^{-1}\ h^{-1}$)
m_S	biomass maintenance coefficient ($g\ S^{-1}\ g\ X^{-1}\ h^{-1}$)
N	stirrer speed (RPM) (s^{-1}) and data number
r_X	rate of biomass production (g cell/h)
$Y_{P/S}$	product yield coefficient based on substrate (g product/g substrate)
$Y_{X/N}$	biomass yield coefficient based on nitrogen (g biomass/g nitrogen)
$Y_{O_2/X}$	oxygen used base on biomass
V_S	superficial air-flow rate (ms^{-1})

Greek letters

δ	exponent coefficient
μ	viscosity of fluid
μ_m	growth specific rate (h^{-1})

water-based paints, etc., due to its superior rheological properties and is used as a rheological control agent in aqueous systems and as stabiliser for emulsions and suspensions. The important properties of the xanthan gum is the ability to form high viscosity solution at low shear forces, highly pseudoplastic, and may also display a viscosity yield value [1]. The xanthan solution is stable over a wide range of salt concentrations (up to 150 g/l NaCl), temperatures (up to 90 °C) and pH (2–11) [2].

The superior properties of xanthan gum have enabled it to compete with most of natural gums, and also become the preferred product due chemical reproducibility and relatively easy supply. The xanthan gum applications have diversified its commercial value, and now become one of the widely used ingredients in food product. As can be seen in Table 1, the concentration of xanthan gum used in food products is very small to enable it

Table 1

Some examples of xanthan gum in food applications

Applications	Uses/benefits	%
Beverages	Provides enhanced body and quality to the reconstituted drink	0.05–0.15
Instant soups	Provide high viscosity in instant soups at both acid and neutral pH	0.30–0.50
Salad dressing	Ideal stabiliser for pourable no-oil, low-oil, and regular salad dressings	0.15–0.50
Cake mixes	Contributes smoothness; air incorporation and retention	0.05–0.25
Sauces	Provide high viscosity in sauces and gravies	0.10–0.30
Relish	Improves the drained weight and virtually eliminates the loss of liquor during handling	0.10–0.25

to confer the required properties without affecting the taste of the final product.

In the agriculture industry, xanthan has been used to improve the flow-ability in fungicides, herbicides, and insecticides formulations by uniformly suspending the solid component [3]. The unique rheological properties of xanthan gum solution also reduce drift, and increase pesticide cling and permanence. Recently, various “tolerance exemptions” were issued by U.S. Environmental Protection Agency for use of xanthan gum as the surfactant in pesticide formulations. Because of its ability to disperse and hydrate rapidly, is non-polluting and gives a good colour yield, xanthan is also used in jet injection printing. Recently, in the formulation of new generations of thermo-set coatings, xanthan gum has been introduced to meet the challenges of producing environmental friendly products.

In the petroleum industry, xanthan gum is used in oil drilling, fracturing, pipeline cleaning, and work-over and completion. Due to xanthan gum is excellent compatibility with salt, and resistance to thermal degradation, it also useful as an additive in drilling fluids. The pseudoplasticity of its solutions would provide low viscosity at the drill bit where the shear rate is high and high viscosity in the annulus where shear is low. Therefore, xanthan would serve a dual purpose by allowing faster penetration at the bit and suspending cuttings in the annulus. For every barrel of oil produced, approximately two remain in the ground. Therefore, enhanced oil recovery (EOR) will be an important use of xanthan gum in the next decades. The basic principle applied is to improve the separation of water and oil thereby would increase oil recovery. However, the quality of xanthan gum is a critical consideration as high impurities would increase the difficulty when refining the oil. Xanthan gum is used in micellar-polymer flooding as a tertiary oil recovery operation. In this application, polymer-thickened brine is used to drive the slug of the surfactant through porous reservoir rock to mobilise residual oil; the polymer prevents bypassing of the drive water through the surfactant band and ensures good area sweeping [4]. In both applications, the function of polymers is to reduce the mobility of injected water by increasing its viscosity.

The other specialty applications employed the xanthan gel is in removing rust, welding rods, wet slag, and cleaning other debris from gas pipelines. Many more application of xanthan gum could be expected to be developed. These wide range

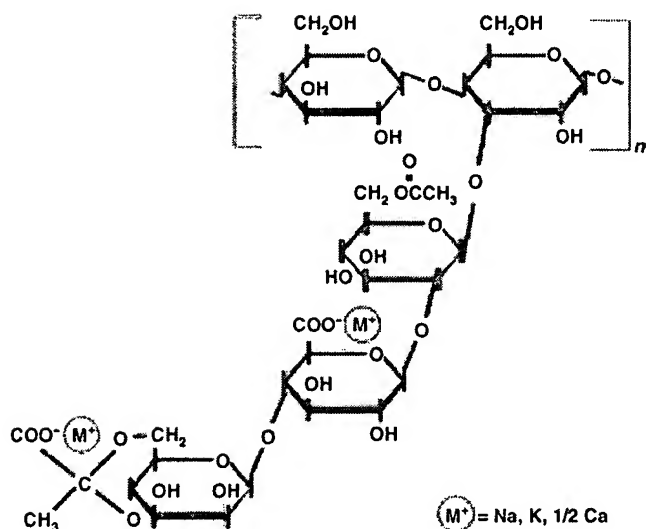


Fig. 1. Structure unit of xanthan gum [49].

of applications of xanthan gum may be summarised due to its superior properties of (1) non-Newtonian behaviour, (2) high viscosity yield even at low concentrations (600–2000 ppm), (3) low sensitivity of viscosity to salinity changes, (4) resistance to mechanical degradation, (5) stable with respect to temperature (up to 90 °C), and (6) a biodegradable material and hence an environmental friendly product [5] (Fig. 1).

3. Chemistry of xanthan gum

Xanthan gum is a complex microbial exo-polysaccharide industrially produced from glucose via fermentation by the plant-pathogenic bacterium, *Xanthomonas campestris* pv. *campestris*. The molecular weight of xanthan gum is approximately 2 million but it can go as high as 13–50 million [6]. As shown in Fig. 1, xanthan gum consists of D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportions of O-acetyl and pyruvyl residues. Xanthan gum is an acidic polymer made up of pentasaccharide subunits, forming a cellulose backbone with trisaccharide side-chains composed of mannose (β 1,4) glucuronic acid (β 1,2) mannose attached to alternate glucose residues in the backbone by α -1,3 linkages. On approximately half of the terminal mannose residues is a ketal linkage joined by a pyruvic acid moiety. Acetyl groups are often present as 6-O substituents on the internal mannose residues. Some external mannoses contain a second 6-O-acetyl substituent [6].

The synthesis of xanthan gum is believed to be similar to exopolysaccharide synthesis by other Gram-negative bacteria [7]. The synthetic pathway can be divided into three parts:

1. Uptake of simple sugars and conversion to nucleotidal derivatives.
2. Assembly of pentasaccharide subunits attached to an isopentyl pyrophosphate carrier.
3. Polymerisation of pentasaccharide repeats units and their secretion.

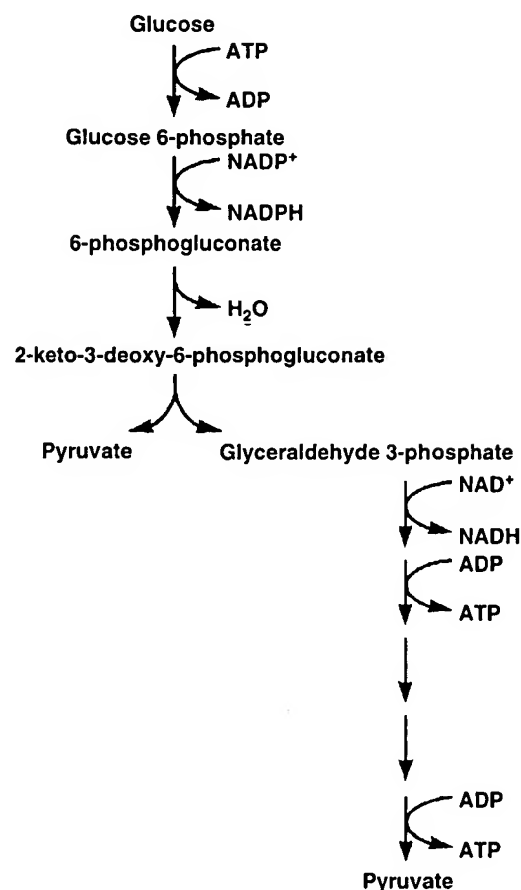


Fig. 2. Entner–Doudoroff pathway [3].

The xanthan backbone is formed by successive additions of D-glucose-1-phosphate and D-glucose from 2 mol of UDP-D-glucose. Thereafter, D-mannose and D-glucuronic acid are added from GDP-mannose and UDP-glucuronic acid, respectively. O-Acetyl groups are transferred from acetyl-CoA to the internal mannose residue, and pyruvate from phosphoenolpyruvate is added to the terminal mannose. Each of these steps requires specific substrates and specific enzymes for completion. If either the substrate or the enzyme is absent, the step will be blocked.

In *X. campestris*, the Entner–Doudoroff pathway in conjunction with the tricarboxylic acid cycle pathway is the predominant mechanism for glucose catabolism (Fig. 2). A small portion of glucose is routed via the pentose phosphate pathway. For glucose uptake, two discrete systems exist. The biosynthesis of xanthan, as in most polysaccharide-producing bacteria, utilises various activated carbohydrate donors to form the polymer on an acceptor molecule. The oligosaccharide repeated units of xanthan are constructed by sequential additions of monosaccharides from sugar nucleotide diphosphates to isoprenoid lipid acceptor molecules. At the same time, acyl substitutes are added from appropriate activated donors. It has been suggested that the construction of the exopolysaccharide follows a “tail-to-head” polymerisation [3]. After the pentasaccharide repeated unit is formed, oligomers are formed by transfer to other lipid intermediates. Oligomer construction normally involves the addition of the longer oligosaccharide sequence to the isoprenoid lipid

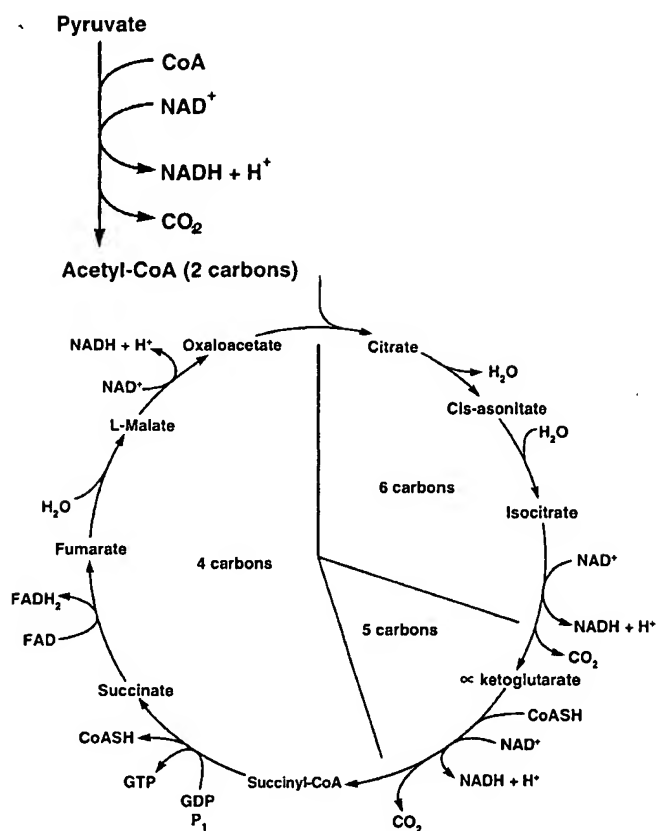


Fig. 3. The tricarboxylic acid cycle [3].

diphosphate. The inactive lipid carrier is dephosphorylated to yield isoprenyl phosphate, which can then re-enter the biosynthetic sequence.

The structure of repeating units is determined by the sequential transfer of different monosaccharides and acyl groups from their respective donors by highly specific sugar transferases, the polymerase enzyme responsible for polymerisation of the pentasaccharides into a macromolecule. The final stages of exopolysaccharide secretion from the cytoplasmic membrane involve passing across the periplasm and the outer membrane and finally excreted into the extracellular environment. This mechanism must exist in all polysaccharide-producing bacteria for releasing polymer from the isoprenoid lipid prior to transport to its final destination (Fig. 3). The process requires an energy source and may be analogous to export lipopolysaccharide to outer membrane in which ATP is the energy supplier [3].

4. Strain of *X. campestris* sp.

The strains for xanthan gum production are selected and improved by several conventional methods. The purpose of genetic modification could be to have improvements of the properties as required by the down stream application, or to suit with the medium supplied, or to improve the product yield, or to improve the performance by reducing the fermentation time, or to simplify the recovering and purification in following processes.

Attempts of mutation of specific genes involved in the xanthan gum synthesis have been made to simplify the repeating unit structure, however the xanthan gum yield was much lower as than that produced from wild-type strains. Betlach et al. [8] have constructed a mutant lacking the glucuronic acid residues and the pyruvate. As result, the xanthan gum solution produced by this strain is highly viscous. Another strategy to alter the structure of xanthan gum is by postsynthetic enzymatic treatment, such as removal of the terminal β -D-glucuronosyl residue from xanthan gum eliminating the mannosyl side-chain terminus. This truncated xanthan gum missing the terminal disaccharide is more viscous than the original xanthan gum [8]. The method of increasing xanthan gum production but varying of the quality of the product of xanthan gum to some extent has been successfully described by Pollock and Thorne [9].

A method of increasing xanthan gum production by some extent was successfully described. Despite positive strain developments, the overall increase of xanthan gum yield by new strains still seems to be unlikely [6]. It may be concluded that modification of the microbial strains to increase the xanthan gum yields is not necessary since the synthetic production for xanthan gum is very efficient with high conversion of carbon sources into a product (50–85%) [10,11]. This may suggest that improvement of xanthan yield and quality could be achieved by improving the fermentor and process design, changing the composition of the medium, and the media feeding strategy.

Due to differing supply of particular raw materials in various part of the world, other attempts have been made by researchers to extend the range of the substrates that can be efficiently used for xanthan gum production. *X. campestris* sp. generally does not use lactose efficiently because of the low level of β -galactosidase activity in the organism. Fu and Tseng [12] had introduced a plasmid carrying β -galactosidase-encoding gene into *X. campestris*. The resulting strain is able to produce xanthan gum in whey-containing medium, but the plasmid is not stable. It was concluded that the unmodified starch used as carbon source in producing xanthan gum may not be a correct method.

5. Nutrients

In order to grow and be reproductive, cells must ingest nutrients necessary to manufacture membranes, proteins, cell walls, chromosomes, and other components. The fact that different cells employ different carbon and energy sources shows clearly that all cells do not possess the same internal chemical machinery. Different growth phase and alteration of the growth medium, for example by using different substrate and limiting nutrients, do not influence the primary backbone structure, but do affect the structure of side-chains, the molecular mass, and the yield, thus xanthan gum produced from a batch culture process would represent a mixture produced at different grow phases and may vary with different culture conditions [13]. As different cultures would require different media and optimum conditions, many studies on nutrients required for the purpose of product side-chain variation and optimisation in xanthan gum biosynthesis have been reported [13–20].

Davidson [13] has demonstrated that by limiting magnesium or phosphate it resulted in the production of low pyruvate containing xanthan gum (Embsden–Meyerhop or EMP). In ammonia-deficient media, high xanthan gum production can be observed. Because of the conventional effects of medium compositions on xanthan gum fermentation, the present industrial xanthan gum production is usually carried out in the medium that provides a compromise between the concentrations needed for cell growth and for xanthan gum formation.

Letisse et al. [20] performed the fermentation using *X. campestris* ATCC 13951 and sucrose as the carbon source. They reported that two nitrogen sources containing either NH_4Cl or NaNO_3 (at 0.055% nitrogen equivalent) showed a slower cell growth rate at 0.07 h^{-1} than ammonium at 0.13 h^{-1} . Yet, the xanthan gum production rates have been increased by nitrate at $0.79\text{ mmol/g cells h}$ cf. ammonium at $0.52\text{ mmol/g cells h}$, although the organic acid content (acetate and pyruvate) of the xanthan gum remained constant at 6.0% and 4.6%, respectively. Ammonium is therefore a better substrate for biomass accumulation, while xanthan gum yields are higher with nitrate used as the nitrogen source. The growth initiation is poor for each of the mineral nitrogen sources, but could be improved using organic nitrogen (e.g. soybean flour hydrolysate). Using mixtures of ammonium and nitrate salts show that ammonium salts are depleted first. Hence, the sequential consumption of the nitrogen sources (soybean hydrolysates, ammonia, and nitrate salt) can be used for further optimisation of the medium. Letisse et al. [20] also mentioned the biomass accumulation and found that it is limited by phosphate availability. A xanthan gum yield of more than 60% (grams of xanthan gum per gram of sugar) can be obtained with constant acetyl content of the xanthan gum. However, pyruvyl substitution would decrease as the growth rate declines due to the metabolic constraints specific to the phosphate depletion. High rates of carbon conversion into xanthan gum can be observed throughout the course of the culture, and the ATP/ADP ratio is not affected by the decline in the specific growth rate. Souw and Demain [15] have reported that sucrose is better substrate for xanthan gum production. They have found that succinate and 2-oxoglutarate have stimulatory effects on xanthan gum production in sucrose-based medium.

Garcia-Ochoa et al. [18] conducted the nutritional study of *X. campestris* NRRL B-1459 for xanthan gum production as a factorial design of experiments and use a statistical tool to deal with optimisation. The concentrations of other nutrients were fixed, e.g. sucrose, calcium, iron, zinc, and citric acid, and the concentration the nutrients nitrogen, magnesium, phosphorus, and sulphur were varied. They found that the xanthan gum concentration over 24 h fermentation time is 10 g/l, higher than usual case of $\sim 7\text{ g/l}$ over the same period reported by other authors [13,15,21,22]. The optimum summary results are shown in Table 2 along with the other findings on the media compositions of four nutrients.

Casas et al. [23] have studied the effects of temperature, initial nitrogen concentration and oxygen mass transfer rate. They have found that the degree of pyruvilation and acetylation and the average molecular weight of the xanthan gum increases with fermentation time for any operating conditions.

Table 2

Comparison of nutrients composition by different authors [18]

Nutrient (g/l)	Davidson [13]	Souw and Demain [15]	Tait et al. [21]	De Vuyst et al. [22]	Garcia-Ochoa et al. [18]
Nitrogen	0.66	0.21	0.66	0.21	0.20
Phosphorus	3.49	3.49	3.49	3.49	2.01
Magnesium	0.02	0.02	0.02	0.02	0.13
Sulphur	0.52	1.50	0.84	1.54	0.07

The highest average molecular weight of xanthan gum molecules are obtained at a 25°C operating temperature, but acetate and pyruvate radical concentrations are the lowest. Nitrogen concentration seems to have no clear effect on the average of the xanthan molecular weight over the conditions studied.

Leela and Sharma [24] studied various types of sugars used as the carbon sources during fermentation of the wild type of *X. campestris* GK6. The obtained xanthan gum yield given as the declining order is glucose, sucrose, maltose, and soluble starch. The result is shown in Table 3.

Lo et al. [25] showed that the preferred course for xanthan gum production is by the two stage batch fermentation using glucose and yeast extract at initial low of 2.5% glucose/0.3% yeast extract for the initiation/exponential phase to a higher level of 5% by adding 2.5% of the glucose during of the steady state phase. There was no further addition of yeast extract. They also reported that by adding the glucose periodically over the course of the fermentation in five equal parts, the yields are poor giving only 18 g/l of xanthan gum. However, using the two stage fermentation technique by a single addition of the additional glucose after 34 h fermentation the yield can reach up to 40 g/l of xanthan gum. The fermentation cycle also takes only 100 h instead of about 120 h using the technique of glucose addition only at the start. Amanullah et al. [11] have extended the sequence feeding approach by introducing the glucose in a series of pulses after the supplied nitrogen had been exhausted in a conversional agitated fermentor. They have found that the yield improved significantly. These two results suggest that a high glucose concentration should be introduced after microbial growth has reached the stationary phase, and although a high glucose concentration is required after reaching the

Table 3

Effect of carbon sources [24]

Carbon sources	Xanthan yield (g/l)
Glucose	14.744
Sucrose	13.234
Maltose	12.321
Fructose	5.232
Xylose	5.531
Arabinose	10.958
Galactose	7.129
Lactose	1.008
Inositol	1.502
Sorbitol	1.401
Soluble starch	12.10
Potato starch	9.754

stationary phase the glucose concentration must be controlled at a level that avoids inhibition by the high concentration of the substrate.

Partially hydrolysed starches that have been utilised in xanthan gum production are from hydrolysed rice, barley, and corn flour [26]. Acid whey [27] sugarcane molasses [28,29], a mixture of mannose and glucose [30], waste sugar beet pulp [31], and peach pulp [32]. Yields and qualities of xanthan gum have been reported to be competitive, however, glucose still give the best in terms of product yield [6,7,24], constancy of supply, and product quality [13]. Some of the possible reasons that could cause low yield and quality are:

- (i) The deficiency of certain functional groups in the carbon sources, resulting in different metabolic pathway reactions being followed. Subsequently, the synthesis produced slightly different structures of extra-cellular polysaccharide (EPs).
- (ii) The nutrients composition vary due different carbon sources used, hence the quality of produced xanthan gum cannot be obtained as required.
- (iii) Formation of other by-products.
- (iv) The components or/and chemical variants in the unmodified starch might become inhibitors.
- (v) A low yield caused by high concentration of non-reacted compounds and lower quality and the post purification process becomes more complicated.

It is concluded that the important findings on nutrients studies are outlined as: a relatively high nitrogen concentration is required for fast cell growth, the type of salt supplied as a source of nitrogen, such as ammonium or nitrate or undefined nitrogen sources resulting in different bacterial growth rates, continuous feeding of the carbon source at certain concentration after microbial reach a steady state growth phase would improve yield, and glucose is a preferable substrate.

6. Batch against continuous process

Although batch culture is commercially preferred having fewer parameters to be controlled and well understood, a problem of operation in batch culture is that the environment for cell growth keeps changing throughout the “growth cycle” and could give adverse conditions, such as toxic products or extreme pH and exhaustion of nutrients. While in continuous culture, the growth medium is continuously supplied to the culture vessel, extreme conditions will not occur as medium is continuously diluted and removed from the vessel. Becker et al. [6] have also pointed out that continuous process shows reasonably high conversion rates of substrate to polymer of 60–70%, but also mention problems of maintaining the sterility and the risks of emergence of fast-growing mutants that do not produce the desired product, xanthan gum. Nevertheless, the continuous process gives a cost competitive system, and with suitable growth conditions considerable yields of polysaccharides can be maintained for more than 2000 h [14], thus the continuous process could be the choice rather than the batch mode.

Although conventional methods can be improved by continuous fermentation, there is still a classic problem that the product contain cells and cell debris, which gives lower the filterability of the xanthan solution and limits its application. The production of cell-free xanthan gum is therefore desirable. In 1966, Esso Production Research Company found that continuous film fermentation reactions can be readily carried out by a continuously depositing a suitable substrate on the surface of a rotating drum or moving belt or similar device, and applying a culture containing selected microorganisms to the film, and then continuously removing the fermentation product after sufficient residence time has elapsed. Tests have shown that such a process makes it possible to use the substrate in higher concentrations, permits surprisingly effective utilisation of the substrate, reduces the time required for carrying out the fermentation reaction, minimises variation in product quality, and simplifies recovery of the fermentation products [26].

7. The xanthan gum kinetics

Selection and development of the appropriate kinetic models particularly for certain microorganisms should begin with understanding of the behaviour and habitat of the microorganisms. Two main options are available for kinetic developments, batch or continuous operation, as the time course of the microbial growth would differ in each operation.

Studies of the unstructured kinetic and the structured kinetics models have been described in a batch process [33–36]. Many authors used the unstructured kinetic to model to describe the synthesis of xanthan gum by *X. campestris* sp. [33,34,37,38]. These unstructured kinetic would include a balance on the cells mass (C_X), the product concentration (C_P), and the substrates concentration (C_S).

A more comprehensive kinetic model could also include the mass transfer limitation caused by increased viscosity, mechanical design of equipment, and variation of the adverse conditions with time, such as cell population density, by which would contribute to increase cells stress and endogenous rate. Garcia-Ochoa et al. [35] have proposed the metabolic structured kinetic model for xanthan gum production by *X. campestris* which is based on the assumptions studied by Pons et al. [39]. The proposed kinetic model is able to describe xanthan gum production at different temperatures and take into account variations in model parameters when the kinetic equations are first order for dissolved oxygen. The latest study by this group [36] proposed a chemical structured kinetic model by involving both carbon source metabolism and nitrogen metabolism into cells. This model considers eight lumped reactions (synthesis of amino acids, both non forming and forming bases, nucleic acids synthesis, both RNA and DNA synthesis, xanthan production, total sugar metabolism, oxidative phosphorylation, and maintenance energy) and eight key compounds (biomass, ammonium, RNA, DNA, intracellular proteins, xanthan gum, sucrose, and dissolved oxygen). The model more closely describes the experimental results and it able to predict the behaviour of the system when some operational conditions are changed, e.g. temperature, initial nitrogen concentration, and also different oxygen trans-

port rates, thus predicting different xanthan production rates by depending on the operational conditions and medium composition (nitrogen source concentration).

As many authors uses unstructured kinetic model and batch processing, the kinetic discussed here is limited to the most used of the kinetic model and the most used of the system. In this case, interest is centred on the population growth rather than the substrate utilisation and the xanthan gum production as both relate to the microbial growth. Other than substrates and nutrients would limit the microbial reproductive is the reactor design, and agitation rates, and changes of viscosity [23]. The general form of microbial growth kinetics may be expressed by Eq. (1).

$$r_X = \frac{dC_X}{dt} \propto f(C_X, C_S, C_P, C_N, C_{O_2}, C_\xi) \quad (1)$$

where r_X is the biomass rate, f the function particular to the system used, C_X the biomass concentration, C_S the substrate concentration, C_P the product concentration, C_N the nitrogen concentration, C_{O_2} the oxygen concentration, and C_ξ is other special adverse components or inhibitors concentrations, e.g. CCO_2 , C_{Poison} .

Weiss and Ollis [38] have expressed growth rate as a function of biomass using the logistic equation which also known as the Verlhurst–Pearl or so called the autonomous equation. The equation can be written as:

$$\frac{dC_X}{dt} = \mu_m C_X \left(1 - \frac{C_X}{C_{X_m}} \right) \quad (2)$$

The modified logistic growth kinetics for describing the batch kinetics of microbial growth during the biosynthesis of extra- and intra-cellular polymers proposed by Mulchandani et al. in a paper published by Luong et al. [33] is given as:

$$\frac{dC_X}{dt} = \mu_m C_X \left(1 - \left(\frac{C_X}{C_{X_m}} \right)^\theta \right) \quad (3)$$

The relationship is valid as long as $[1 - (C_X/C_{X_m})^\theta]$ in non-negative, i.e., $\theta > 0$. The constant θ could be defined as an index of inhibitory effects that accounts for the deviation of growth from the exponential growth. For a very large θ , the generalised logistic equation kinetic approaches the exponential growth equation:

$$\frac{dC_X}{dt} = \mu_m C_X \quad (4)$$

Luong et al. [33] proposed a combination of Monod and logistic or modified logistic as follows:

$$\frac{dC_X}{dt} = \mu_m \frac{C_S}{K_S + C_S} \left(1 - \frac{C_X}{C_{X_m}} \right) C_X \quad (5)$$

The two substrate evolutions with time are expressed in term of stoichiometric coefficients [34].

$$\frac{dC_S}{dt} = -\frac{1}{Y_{P/S}} \frac{dC_P}{dt} \quad (6)$$

$$\frac{dC_N}{dt} = -\frac{1}{Y_{X/N}} \frac{dC_X}{dt} \quad (7)$$

where $Y_{P/S}$ is the product yield coefficient based on substrate, $Y_{X/N}$ is the biomass yield coefficient based on nitrogen. Unfortunately for the biomass in xanthan production, the rate is not of the Monod type, therefore the equation proposed by Luong et al. [33] could not suitable to represent the synthesis of xanthan gum. Garcia-Ochoa et al. [34] have proposed the rates as follows:

$$\frac{dC_X}{dt} = k_X C_N C_X \quad (8)$$

$$\frac{dC_P}{dt} = k_P C_S C_X \quad (9)$$

The logistic equation is given from the combination of Eqs. (7) and (8).

$$\frac{dC_X}{dt} = k_X \frac{C_{X_m}}{Y_{X/N}} C_X \left(1 - \frac{C_X}{C_{X_m}} \right) \quad (10)$$

When nitrogen is the limiting factor, microbial growth has ceased after the nitrogen source had exhausted, therefore the parameter C_{X_m} is replaced by:

$$C_{X_m} = C_{X_0} + Y_{X/N} C_{N_0} \quad (11)$$

$$\frac{dC_X}{dt} = k_X \left(\frac{C_{X_0}}{Y_{X/N}} + C_{N_0} \right) C_X \left(1 - \frac{C_X}{C_{X_0} + Y_{X/N} + C_{N_0}} \right) \quad (12)$$

Carbon source is used for maintenance and for growth, thus:

$$\frac{dC_S}{dt} = -m_S C_X - \frac{1}{Y_{X/S}} \frac{dC_X}{dt} \quad (13)$$

Dissolve oxygen is described by the following equation:

$$\frac{dC_{O_2}}{dt} = k_L a (C_{O_2}^* - C_{O_2}) - \left(m_{O_2} C_X + \frac{1}{Y_{O_2/X}} \frac{dC_X}{dt} \right) \quad (14)$$

Where the oxygen mass transfer coefficient was given as:

$$k_L a = 3.08 \times 10^{-4} V_S^{0.43} N^{1.75} \mu^{-0.39} \quad (15)$$

So far from the unstructured kinetics model have been reviewed, no authors have evaluated the microbial growth causes by the adverse condition e.g. carbon dioxide contents and other inhibits chemical produced by the microbial itself. However, a modified of the combination logistic and Monod kinetic given by Luong et al. [33] (shown in Eq. (16)) which represents the cell growth kinetic of *A. eutrophus* sp., has described the effect of substrate concentration to the growth rate. The inhibition effects consideration proposed by Mulchandani et al. might partially describe adverse conditions, and if so, a combination of the substrate concentration effect (Eq. (16)) and inhibition effects (Eq. (17)) into the microbial kinetic rate suggested by Garcia-Ochoa et al. [34] could completely describe the adverse conditions effect to the microbial growth rate.

$$r_X = \mu_m \frac{C_S}{K_S + C_S} \left(1 - \frac{C_S}{C_{S_m}} \right)^\delta C_X \quad (16)$$

$$r_X = \mu_m \frac{C_S}{K_S + C_S} \left(1 - \left(\frac{C_X}{C_{X_m}} \right)^\theta \right) C_X \quad (17)$$

$$\frac{dC_X}{dt} = k_X \left(\frac{C_{X_0}}{Y_{X/N}} + C_{N_0} \right) C_X \left(1 - \left(\frac{C_X}{C_{X_0} + Y_{X/N} + C_{N_0}} \right)^\theta \right) \times \left(1 - \frac{C_S}{C_{S_m}} \right)^\delta C_S \quad (18)$$

In this kinetic review, the function of microbial growth is proposed as given in Eq. (1). The effects of (a) carbon source is given by Eqs. (6) and (13), (b) biomass is given by Eq. (11), (c) nitrogen source is given by Eq. (11), (d) oxygen is given by Eq. (14), and (e) the proposed Eq. (18) which has considered other adverse conditions and substrate inhibition.

8. Commercial productions processes

Most commercial production of xanthan gum uses glucose or invert sugars, and most industries prefer batch instead of continuous [7,20,24]. Quality assurance and easier of control are reasons why the xanthan gum production uses invert sugars, instead of polysaccharides, and batch process instead of continuous operation.

A typical commercial production process starts with inoculums of *X. campetris* that are prepared in suitable fermentation medium in conventional batch processing using mechanically agitated vessels. The aerated culture that undergoes aerobic process is held at the following operating conditions: temperature approximately $T=28-30^\circ\text{C}$, $\text{pH} \sim 7$, the aeration rate must higher than 0.3 (v/v), and the specific power input for agitation higher than 1 kW/m^3 [3]. The fermentation process is carried out for about 100 h and converts an approximately 50% of the glucose into the product. Inoculums preparation includes several stages which require a set of the reactor ranging from 10 l for the initial seed up to 100 m^3 in production stage by which the volume is usually enlarged by 10 folds. As the fermentation evolves, cells would grow exponentially resulting in rapid consumptions of the nitrogen source. After fermentation stage, multi steps downstream processes would follow. Fig. 4 shows an example of the xanthan gum process used by industry that includes multi steps of downstream process.

When industrial grade xanthan is required, the post fermentation process treatment may be started with pasteurisation on the fermented broth to sterilise the bacterial and to deactivate the enzymes. This process usually uses a large amount of alcohol to precipitate the xanthan gum, and the precipitated xanthan gum is then sprayed dry or maybe re-suspended on the water and then re-precipitated. When cell-free xanthan gum is required, cells

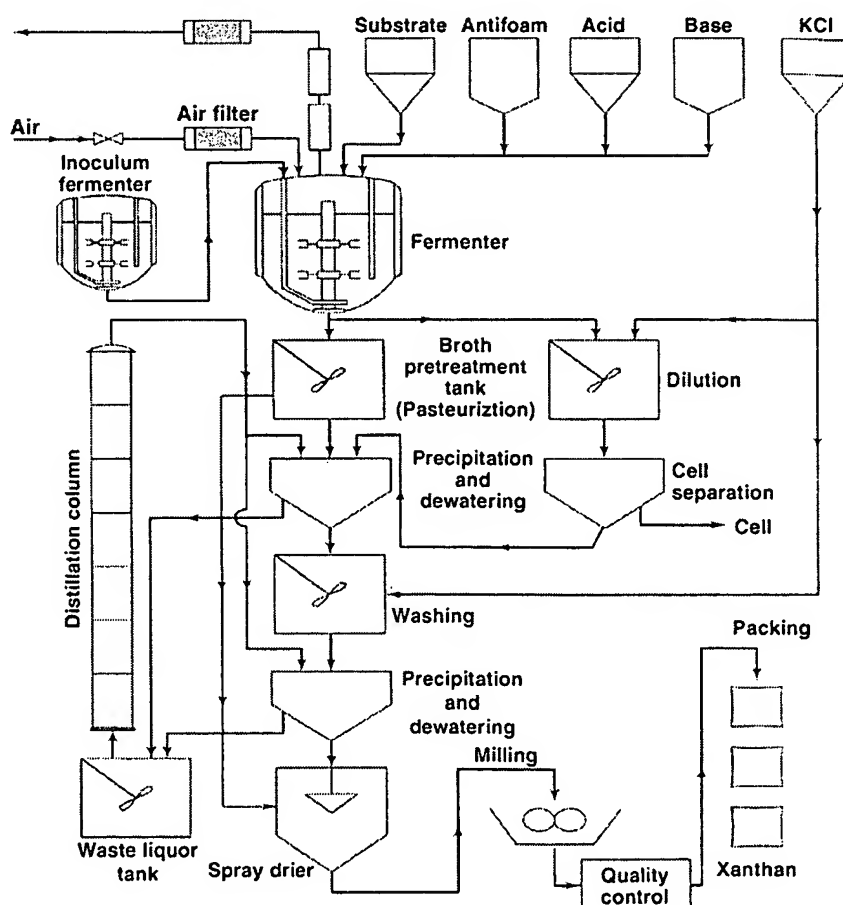


Fig. 4. Flow sheet of xanthan production in conventional stirred tank fermentor.

centrifugation is facilitated by diluting the fermentation broth to improve the cell separation.

The cell separation by dilution process from highly viscous xanthan solution is a cost-intensive process [40]. A favoured method is by adding alcohol and adding the salt would improve precipitation by creating reverse effect charges. The xanthan gum obtained in wet solid form would undergo the dewatering and washing to obtain the final purity required. Alcohol used for xanthan precipitation is recovered by distillation column. Washing may be carried out to improve the quality of product hence it would entirely remove particulate matters, such as cell debris, microgels, organic residues and pigments. Concentrated xanthan gum is then re-dissolved and washed with water/KCl to reduce viscosity, precipitated and dewatered again until satisfied with the produced quality. Finally, the precipitated xanthan gum is spray-dried in batch or continuous driers. The dry xanthan gum is milled to the desired mesh sizes for control of disperse ability and dissolution rate as well as to get the handling much easier.

9. Recent developments and future studies

Development and improvement have been studied for the xanthan production [11,19,25]. In the last few years, the membrane processes have been increasingly used for concentrating of high viscous broth [41–44]. Ultrafiltration was also reported that would save up to 80% the energy required for recovering of xanthan gum from the fermentation [43].

Lo et al. [42] studied the performance of the ultrafiltration system by the whole fermentation broth, introducing the heat treatment before exposing to the ultrafiltration, and the cell free broth collected from immobilised cells fermentation. Results revealed that the membrane was heavily fouled by cells when the whole broth was introduced. When heat treatment was introduced in the first place to lyses the cells, ultrafiltration performance was improved but the cell debris still contributed a significant membrane fouling. This could also become a major problem in a long-run operation. The cell free xanthan broth was not notable fouled the membrane during the entire period.

As the cell free xanthan gum has eliminated the membrane fouling, Yang et al. [45] have developed a novel of centrifugal packed-bed reactor (CPBR) used for viscous xanthan gum production. *X. campestris* cells were immobilised in a rotating fibrous matrix by natural attachment to the fibre surfaces. Continuously pumping and circulating the medium broth through the rotating fibrous matrix would ensure a good transfer of the gas and liquid with the cells. Fibrous matrix support would give a good separation of the xanthan from the cells has said immobilised most cells onto the fibre surfaces. Yang et al. [46] have also studied a good device for cells adsorption used as a matrix support. Attempting four different woven materials; cotton towel, cotton fabric, and 50% cotton and 50% polyester, the result showed that cotton with rough surfaces is the preferred material. Cell adsorption to cotton was also reported faster than polyester fibres, and almost all cells have been removed from the fermentation broth. They also found that cells adsorption is not efficient in the absent of xanthan gum [46]. The CPBR and

cells adsorption method have potential to be developed further for continuous operation.

Other developments are the axial-flow hollow fibre cell culture bioreactors [47], the fibrous-bed bioreactor for continuous production of developmental endothelial locus-1 by osteosarcoma cells [48], and the ceramic membrane reactor which possibly help in development of the continuous xanthan gum production.

10. Conclusion

Many attempts have been reported for optimising variables of the xanthan gum fermentation, i.e. the nutrient composition and feeding technique, temperature, pH, agitation, and adding antifoam [11,13–20,23,25,34–36]. Some attempts also have been made to use immobilised-cell cultures for production of xanthan gum [45,46] and so to other bacterial polysaccharides [50,51]. All shows some improvement in the area studied. Other substrates were also tested [26–29] but *glucose is still the best in-term of the product yield*, supply, and the product quality [2,6,7,13]. Studies of the unstructured kinetic and the structured kinetics models have also been described in batch processes [33–36] but yet none of studies found reviewed so far is working on the continuous kinetic model. In general conclusion, most of previous works are not attempted to give a huge impact on the price of xanthan gum but more on fine tunings on the particular areas studied, thus it is the task of this review to initiate a new strategy on the xanthan gum processing technology that could improve the quality, increase the productivity and also reduce the cost.

Looking typically on the conversional xanthan production, glucose powder purchased from local factories would be diluted to 20–50 g/l and then sterilised along with other ingredients. This ingredient is fermented in bioreactor by batch or continuous means, and also by several feeding techniques. Mixing is the main problem in batch fermentations as the produced broth during the production stage is very viscous that require considerable balance between cell disruptions and oxygen transfer. Thus, it suggests that the xanthan gum in the liquid phase should be continuously removed which could be done by cells adsorption and using membrane technology. This strategy could solve mixing problems and fouling.

Giving the support, e.g. cotton wool and fabric, for microorganisms to adsorb may ensure the nature physical separation between microorganisms and the liquid phase containing nutrients and products. But the specific xanthan productivity was reported lower in centrifugal fibrous-bed bioreactor than in STR that is because of relatively low cell viability (~60%) and it is limited by oxygen transfer in the CPBR [45]. Some xanthan gum is also required for cell adsorption onto the support [46], thus a complete removal of the xanthan gum would decrease the rate of cells adsorption and may also increase fouling to the membrane. Thus, this has further suggested that only excess xanthan gum should be removed from the bioreactor. The process should be more effective by recycling the medium across the membrane only during the production stage, which could also avoid membrane fouling caused by cells.

The problem of the limited oxygen transfer could require a new design of the bioreactor by simulating the environment nature where the microbe attaches in the static place (cabbage plant). It may be designed by allowing a freely moving of liquid media and air passing through the porous fibrous matrix to ensure a good contact with the cells that adsorb on the fibrous matrix support. This strategy may improve the oxygen transfer, while microbes would continuously get a fresh medium, thus could increase reaction rate and also reduce mutation problems.

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References

- [1] Yoshida T, Tanner RD. Bioproducts and bioprocess, vol. 2. Berlin, Heidelberg, Germany: Springer-Verlag; 1993.
- [2] Lee BH. Fundamentals of food biotechnology. United States: VCH Publishers Inc.; 1996.
- [3] Flickinger FC, Drew SW. Encyclopedia of bioprocess technology: fermentation, vol. 5; 1999. p. 2706–7.
- [4] Byong HL. Fundamentals of food biotechnology. United States: VCH Publishers Inc.; 1996.
- [5] Malik VS, Sridhar P. Industrial biotechnology. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.; 1992.
- [6] Becker A, Katzan F, Puhler A, Ielpi L. Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl Microb Biotechnol 1998;50:145–52.
- [7] Harding NE, Cleary JM, Luis L. Genetics and Biochemistry of Xanthan Gum Production by *Xanthomonas campestris*. Dlm. In: Hui YH, Khachatourians G, editors. Food technology microorganisms. USA: John Wiley-VCH Inc.; 1995. p. 495–514.
- [8] Betlach MR, Capage DH, Doherty DH, Hassler RA, Henderson NM, Vanderslice RW, et al. Genetically engineered polymers: manipulation of xanthan gum biosynthesis. In: Yalpani M, editor. Industrial polysaccharides genetic engineering, structure/property relations and applications. Amsterdam: Elsevier Science Publisher B.V.; 1987. p. 35–50.
- [9] Pollock, Thorn. A method of increasing xanthan gum production and decorating the product. US Patent Number 233019A2; 1987.
- [10] Linton JD. The relationship between metabolite production and the growth efficiency of the producing organism. FEMS Microbiol Rev 1990;75:1–18.
- [11] Amanullah A, Satti S, Nienow AW. Enhancing xanthan fermentations by different modes of glucose feeding. Biotechnol Prog 1998;14:265–9.
- [12] Fu J-F, Tseng YH. Construction of lactose-utilising *Xanthomonas campestris* and production of xanthan gum from whey. Appl Environ Microbiol 1990;56:919–23.
- [13] Davidson W. Production of polysaccharide by *Xanthomonas campestris* in continuous culture. FEMS Microb Lett 1978;3:347–9.
- [14] Evans CGT, Yeo RG, Ellwood DC. Continuous culture studies on the production of extracellular polysaccharides by *Xanthomonas juglandis*. In: Berkeley RCW, Gooday GW, Ellwood DC (pny.). Microbial polysaccharides and polysaccharases. London: Academic Press Inc. (London) Ltd.; 1967. p. 51–67.
- [15] Souw P, Demain AL. Nutritional studies on xanthan gum production by *Xanthomonas campestris* NRRL B1459. Appl Environ Microbiol 1979;37:1186–92.
- [16] Vashitz O, Sheintuch M. Analysis of polymer synthesis rates during steady state growth of *X. campestris*. Biotechnol Bioeng 1991;37:383–5.
- [17] Zhang, Greasham. Chemically defined media for commercial fermentations. Appl Microbiol Biotechnol 1999;51:407–21.
- [18] Garcia-Ochoa F, Santos VE, Fritsch AP. Nutritional study of *Xanthomonas campestris* in xanthan gum production by factorial design of experiments. Enzyme Microb Technol 1992;14:991–7.
- [19] Garcia-Ochoa F, Gómez Castro E, Santos VE. Oxygen transfer and uptake rates during xanthan gum production. Enzyme Microb Technol 2000;27:680–90.
- [20] Letisse F, Chevallereau P, Simon JL, Lindley ND. Kinetic analysis of growth and xanthan gum production with *Xanthomonas campestris* on sucrose, using sequentially consumed nitrogen sources. Appl Microb Biotechnol 2001;55:417–22.
- [21] Tait MI, Sutherland IW, Sturman C. Effect of growth conditions on the production, composition and viscosity of *Xanthomonas campestris* exopolysaccharide. J Gen Microb 1986;132:1483–92.
- [22] De Vuyst L, Vermiere A, Van Loo J, Vandamme EJ. Nutritional, physiological and process-technological improvements of xanthan gum fermentation process. Mec Fac Landbouww Rijkuniv Gent 1987;52:1881–900.
- [23] Casas JA, Santos VE, Garcia-Ochoa F. Xanthan gum production under several operational conditions: molecular structure and rheological properties. Enzyme Microb Technol 2000;26:282–91.
- [24] Leela JK, Sharma G. Studies on xanthan production from *Xanthomonas campestris*. Bioprocess Eng 2000;23:687–9.
- [25] Lo YM, Yang ST, Min DB. Effects of yeast extract and glucose on xanthan production and cells growth in batch culture of *Xanthomonas campestris*. Appl Microbiol Biotechnol 1997;47:689–94.
- [26] Glicksman M. Gum technology in the food industry. New York: Academic Press Inc.; 1975.
- [27] Charles M, Radjai MK. Xanthan gum from acid Whey. Sandford: Dlm.; 1977.
- [28] El-Salam A, Fadel MA, Murad HA. Bioconversion of sugarcane molasses into xanthan gum. J Biotechnol 1993;33:103–6.
- [29] Kalogiannis S, Iakovidou G, Maria LK, Kyriakidis DA, Skaracis GN. Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. Process Biochem 2003;1–8.
- [30] Jean-Claude MGT, Roland HFB, Benedicte LTW. Production of xanthan gum by fermenting a feedstock containing a mixture of mannose and glucose. Biotechnol Adv 1997;15(1):267.
- [31] Yoo SD, Harcum SW. Xanthan gum production from waste sugar beet pulp. Bioresour Technol 1999;70(1):105–9.
- [32] Papil RM, Ekateriniadoul LV, Beletsiotis E, Typas MA, Kyriakidis DA. Xanthan gum and ethanol production by *Xanthomonas campestris* and *Zymomonas mobilis* from peach pulp. Biotechnol Lett 1999;21(39–43):39.
- [33] Luong JHT, Mulchandani A, Leduy A. Kinetics of biopolymers synthesis: a revisit. Enzyme Microb Technol 1998;10:326–33.
- [34] Garcia-Ochoa F, Santos VE, Alcon A. Xanthan gum production: an unstructured kinetic model. Enzyme Microb Technol 1995;17:206–17.
- [35] Garcia-Ochoa F, Santos VE, Alcon A. Metabolic structured kinetic model for xanthan gum production. Enzyme Microb Technol 1998;23:75–82.
- [36] Garcia-Ochoa F, Santos VE, Alcon A. Chemical structured kinetic model for xanthan gum production. Enzyme Microb Technol 2004;35:284–92.
- [37] Moraine RA, Rogovin P. Xanthan biopolymer production at increased concentration by pH control. Biotechnol Bioeng 1971;13:381–91.
- [38] Weiss RM, Ollis DF. Extracellular microbial polysaccharides. Substrate, biomass, and product kinetic equations for batch xanthan gum fermentation. Biotechnol Bioeng 1980;22:859–73.
- [39] Pons A, Dussap CG, Gros JB. Modelling *Xanthomonas campestris* batch fermentation in bubble column. Biotechnol Bioeng 1989;33:394–405.
- [40] Balows A, Trüper HG. The prokaryotes. New York: Springer-Verlag; 1991.
- [41] Pritchard M, Howell JA, Field RW. The ultrafiltration of viscous fluids. J Membr Sci 1995;102:223–35.
- [42] Lo YM, Yang ST, Min DB. Kinetic and feasibility studies of ultrafiltration of viscous xanthan gum fermentation broth. J Membr Sci 1996;117:237–49.

- [43] Lo YM, Yang ST, Min DB. Ultrafiltration of xanthan gum fermentation broth: process and economic analyses. *J Food Eng* 1997;31(February (2)):219–36.
- [44] Howell JA, Field R, Wu D. Ultrafiltration of high-viscosity solutions: theoretical developments and experimental findings. *Chem Eng Sci* 1996;51(9):1405–15.
- [45] Yang ST, Lo YM, Min DB. Xanthan gum fermentation by *Xanthomonas campestris* immobilized in a novel centrifugal fibrous-bed bioreactor. *Biotechnol Prog* 1996;12:630–7.
- [46] Yang ST, Lo YM, Chattopadhyay D. Production of cell-free xanthan fermentation broth by cell adsorption on fibers. *Biotechnol Prog* 1998;14:259–64.
- [47] Brotherton JD, Chau PC. Modeling of axial-flow hollow fiber cell culture bioreactors. *Biotechnol Prog* 1996;12:575–90.
- [48] Chen C, Huang YL, Yang ST. A fibrous-bed bioreactor for continuous production of developmental endothelial locus-1 by osteosarcoma cells. *J Biotechnol* 2002;97(1):23–39.
- [49] Sutherland IW. Microbial exopolysaccharide synthesis. Dlm. In: Sandford PA, Laskin A, editors. *Extracellular microbial polysaccharides*. USA: American Chemical Society; 1977. p. 40–57.
- [50] Saude N, Chèze-Lange H, Beunard D. Alginate production by *Azotobacter vinelandii* in a membrane bioreactor. *Process Biotechnol* 2002;38: 273–8.
- [51] Kalogiannis S, Gesthimani I, Maria LK, Dimitrios AK, George NS. Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses 2003;39:249–56.